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Haemophilia

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Abstract | Haemophilia A and B are rare congenital, recessive X-linked disorders caused by lack or deficiency of clotting factor VIII (FVIII) or IX (FIX), respectively. The severity of the disease depends on the reduction of levels of FVIII or FIX, which are determined by the type of the causative mutation in the genes encoding the factors (*F8* and *F9*, respectively). The hallmark clinical characteristic, especially in untreated severe forms, is bleeding (spontaneous or after trauma) into major joints such as ankles, knees and elbows, which can result in the development of arthropathy. Intracranial bleeds and bleeds into internal organs may be life-threatening. The median life expectancy was ~30 years until the 1960s, but improved understanding of the disorder and development of efficacious therapy based on prophylactic replacement of the missing factor has caused a paradigm shift, and today individuals with haemophilia can look forward to a virtually normal life expectancy and quality of life. Nevertheless, the potential development of inhibitory antibodies to infused factor is still a major hurdle to overcome in a substantial proportion of patients. Finally, gene therapy for both types of haemophilia has progressed remarkably and could soon become a reality.

Haemophilia A and haemophilia B are congenital disorders caused by deficiency or absence of either of two coagulation proteins, factor VIII (FVIII) for haemophilia A (encoded by *F8*) and factor IX (FIX) for haemophilia B (encoded by *F9*). Deficiency of coagulation factor XI (FXI) has been defined as haemophilia C in the past; however, currently only haemophilia A and B are defined as haemophilia, and all other clotting factor deficiencies are referred to as rare bleeding disorders. Degree of severity is determined by the type of causative mutation. Both haemophilia A and haemophilia B are X-linked recessive disorders and affect almost exclusively men and boys[1]. Women are usually heterozygous carriers of one mutated gene and may present with reduced FVIII or FIX levels, usually associated with mild symptoms. The classification of the severity of haemophilia is based on the amount of residual FVIII or FIX activity: severe (<1 international unit (IU)/dl), moderate (1–5 IU/dl) or mild (6 IU/dl to <40 IU/dl)[2,3]. Haemophilia A and B have similar symptoms and are both characterized by bleeding, especially into large joints such as elbows, knees and ankles (the index joints); such joint bleeding eventually causes painful and disabling haemophilic arthropathy. However, rare, life-threatening bleeds (for example, intracranial bleeds and bleeds in other internal organs) may occur, regardless of the severity of the disease, but especially in the severe forms[4]. In severe haemophilia, spontaneous bleeds are common, whereas persons with moderate or mild haemophilia have a milder phenotype, although serious bleeding may occur in connection with injuries and surgery. Treatment of haemophilia has improved substantially during recent decades, owing to the availability of efficacious and safe clotting factor concentrates that can be given as long-term prophylaxis from early childhood in the more severe cases. Before the era of safe blood products, blood-transmitted disorders such as hepatitis C virus and HIV infections affected large proportions of people with haemophilia who were treated with commercially available pooled plasma-derived clotting factor concentrates[4]. The advent of comprehensive care centres in the 1970s and home infusion of replacement factor considerably improved outcomes. However, in low-resource settings progress is slow, and globally the majority of people with haemophilia still do not have access to appropriate treatment[5]. Development of inhibitory antibodies to FVIII or FIX as a result of factor replacement therapy still constitutes a major threat to the health of people with haemophilia[6]. New improved therapies are entering the market and hold promise for the near future. These treatments include FVIII and FIX concentrates that are modified to have improved pharmacokinetics with longer half-life, and non-factor-based haemostatic agents[7,8]. Gene therapy is now a reality, on the basis of very promising results from several clinical trials[9,10]. In this Primer, we describe state of the art basic and clinical knowledge of the disorder and the recent breakthroughs in treatment options. The dream of a 'healthy' person living with haemophilia seems to be close.

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https://doi.org/10.1038/s41572-021-00278-x

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Epidemiology

Haemophilia prevalence is determined by life expectancy and access to treatment[11,12], and accurate data on the epidemiology are difficult to obtain. Haemophilia affects all ethnic groups equally, with haemophilia A occurring more commonly than haemophilia B[13]. Data on incidence and prevalence rely on adequate diagnosis and registration, as well as on access to treatment. In particular, access to treatment and treatment intensity are determinants of life expectancy[11,14]. A recent report from the Netherlands, a country using intensive treatment, showed that persons with haemophilia had a 6-year reduction in overall life expectancy[11,14].

The proportions of individuals with severe, moderate or mild disease, as well as the incidence and prevalence of haemophilia, are most accurately established in data from countries with national registries[13]. Ideally, all patients with this rare condition should be included in a national registry. This will enable better planning and evaluation of care[15]. The most reliable estimates come from the UK, with a prevalence of 12.8 per 100,000 males for haemophilia A and 2.7 per 100,000 males for haemophilia B[16]. For haemophilia A, approximately 40% of patients have severe disease, approximately 10% moderate disease and the remaining approximately 50% of individuals have mild haemophilia[16]. For haemophilia B, this distribution is less established. In the 2018 annual report from the European Haemophilia Safety Surveillance (EUHASS) registry, the proportion of severe haemophilia A was 7,214 of 17,815 individuals (40.5%; 95% CI 39.8–41.2%), and for haemophilia B 1,229 of 3,815 (32.2%; 95% CI 25.0–54.5%)[17]. The most recent report from the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO)[17] reports slightly different distributions for both 6,819 patients with haemophilia A (30.2% severe disease, 11.9% moderate and 57.0% mild) and 1,836 patients with haemophilia B (24.0% severe disease, 22.7% moderate and 53.0% mild). The UK data for haemophilia B are affected by a founder effect in Ireland, leading to an increased proportion of patients with moderate disease[16].

The best estimate for the incidence of haemophilia is provided by the long-standing Haemophilia Registry established in the UK in 1974. In 2015, the UK reported 193 new cases of haemophilia A in a male population of 32 million (including 61 patients with severe and 17 with moderate haemophilia), resulting in an incidence of 0.6 per 100,000 males for haemophilia A overall, and 0.19 per 100,000 males for severe haemophilia A[16]. For haemophilia B, the UK reported 38 new cases (11 severe, 8 moderate and 19 mild disease), resulting in an incidence of 0.19 per 100,000 males for haemophilia B overall and 0.034 per 100,000 males for severe haemophilia B.

The prevalence of haemophilia A is commonly reported as 1 in 5,000 in the male population and 1 in 10,000 overall. However, more precise estimates clearly show the effects of access to diagnosis, registration and treatment. In a report on 106 countries, the prevalence of haemophilia A was estimated at 12.8 per 100,000 males in high-income countries but only 6.6 per 100,000 males in low-income countries[13]. The effects of improved registration, and potentially treatment, are clearly visible in the UK data: the prevalence of haemophilia A increased from 9.3 per 100,000 males at the start of the national registry in 1974 to 21.6 per 100,000 males in 2006 (REF.[13]). Owing to a reduced life expectancy in patients with no or limited access to treatment, younger patients are over-represented in many low-income countries[11,12].

The prevalence of haemophilia B is commonly reported as one in 30,000 males. Again, more precise estimates are affected by access to treatment and registration. A review of data from 105 countries reported a prevalence of 2.7 per 100,000 males in high-income countries and 1.2 per 100,000 males in low-income countries. Strikingly, three countries (Ireland, Macedonia and Hungary) showed a significantly increased prevalence of haemophilia B, probably secondary to a founder effect[18].

Data on the prevalence of women carriers of one mutated copy of *F8* or *F9* who classify as having haemophilia (that is, with clotting factor activities of <40 IU/dl) are scarce. Although some national registries have started to collect these data, these women are at risk of underdiagnosis and undertreatment, as illustrated by a Dutch study reporting that the median age at the time of testing was 30 years and that 31% of women with a positive family history of haemophilia were unaware of their carrier status at the time of delivering a child with haemophilia[19]. The most recent UKHCDO report[17] included 714 (10.5%) women with FVIII <40% and only 35 (2.3%) women with FIX <40%.

Mechanisms/pathophysiology

The lay understanding of haemophilia is of an excessive bleed risk, either in response to trauma, however minor, or spontaneously, most notably into joints, muscles or intracranially. Anecdotal experience of now elderly persons with haemophilia who underwent dental extractions in their childhood encapsulates the pathophysiology underlying this bleed risk. In the absence of treatment, a large, friable clot would form in the dental socket but repeatedly fail to stop bleeding over a period of days or weeks, necessitating prolonged hospital stays. The discovery that haemophilia A and B are the result of deficiencies of separate clotting factors, FVIII or FIX, respectively, dates to 1952 (REF.[20]). FVIII and FIX contribute to a complex cascade of clotting proteins culminating in the formation of a robust fibrin clot at the specific site of bleeding (FIG. 1). Haemostasis is initiated upon a breach of the endothelium of blood vessels, enabling

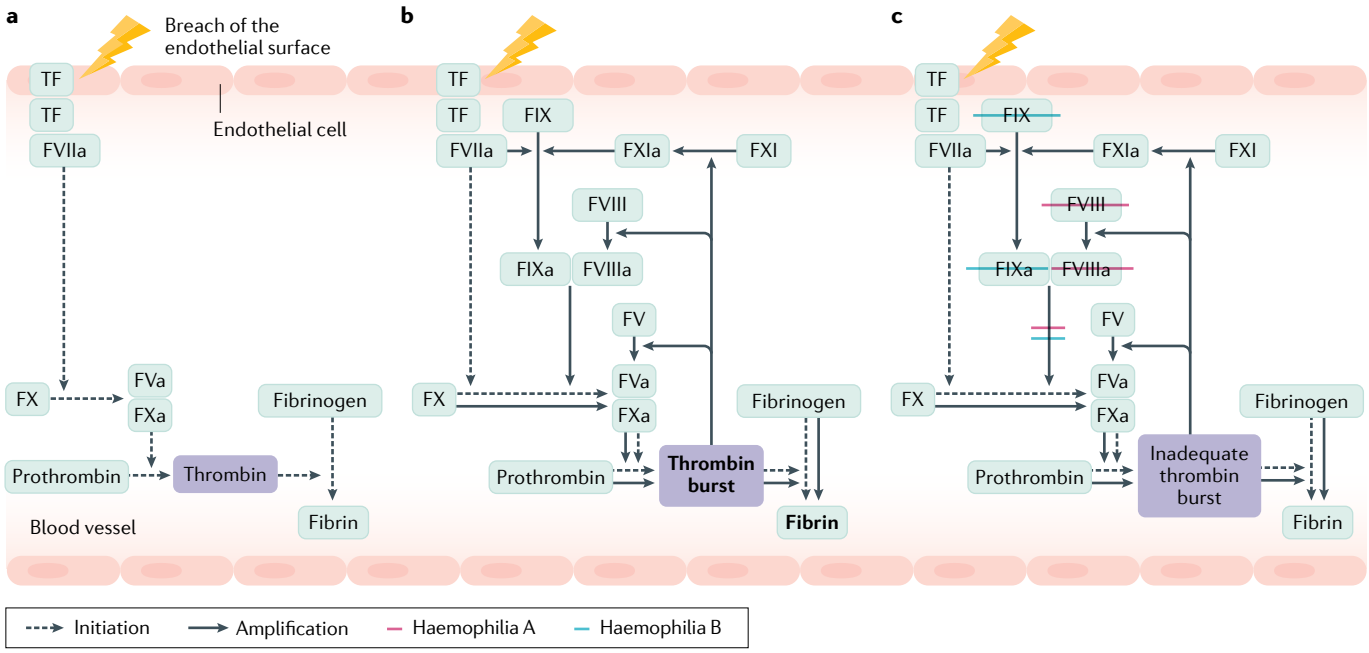

Fig. 1 | **Initiation and amplification of the clotting cascade. a** | Blood vessel injury after trauma or surgery enables tissue factor (TF) contact with circulating activated factor VII (FVIIa), generating early thrombin production. **b** | Thrombin recruits additional enzymatic complexes to amplify the thrombin burst to ultimately enhance clot strength. **c** | Deficiency of factor VIII (FVIII) or factor IX (FIX) in haemophilia A or B, respectively, results in underproduction of thrombin in the clotting cascade. This inadequate thrombin burst compromises the ability to form a robust fibrin clot at the point of need, resulting in a bleeding tendency[21,27,45]. FVa, activated factor V; FIXa, activated factor IX; FXa, activated factor X.

tissue factor (TF) to contact and activate FVII. Activated FVII (FVIIa) initiates a limited cascade, independently of FVIII and FIX, producing modest amounts of thrombin (FIG. 1a). Thrombin is a crucial component of this early clotting process, which includes activating platelets to aggregate and plug the collagen-exposed subendothelial surface, facilitated by von Willebrand factor (vWF), slowing the flow of blood (primary haemostasis) and also converting small amounts of platelet-bound fibrinogen into fibrin. The phospholipid membranes of these adherent platelets are the surface on which the subsequent cascade of clotting factors, including FVIII and FIX, is anchored, facilitating amplification and subsequent propagation of thrombin production to effect a 'thrombin burst'[21] (FIG. 1b). During this 'secondary haemostasis', increased amounts of fibrinogen are converted into fibrin to stabilize the forming thrombus.

FVIII and FIX become activated in this amplification step, forming a complex to activate FX (FIG. 1b). Deficiency of either FVIII or FIX results in compromised FX activation, resultant suboptimal thrombin generation and consequent inadequacy of early clot strength. Thus, the pathophysiological bleed risk of haemophilia is a result of inadequate thrombin generation (FIG. 1c) due to upstream clotting factor deficiency[21].

Haemophilia A

F8. The FVIII-coding gene (*F8*) consists of 26 exons. Positioned at the distal end of the X chromosome long arm (Xq28), it encodes the mature 2,332 amino acid FVIII protein[22]. Defective *F8* results in haemophilia A, an X-linked recessive inherited bleeding disorder. Both haemophilia A and B arise as de novo mutations in approximately 30–50% of cases[4], despite assumptions that there must be a family history, as exemplified by the many generations of individuals affected by haemophilia in European royal families[23,24]. Thus, a negative family history is insufficient to exclude haemophilia when clinical presentation is suggestive of an excessive bleed tendency. Additionally, it is now recognized that a substantial subgroup of women and girls carrying a defective *F8* gene have reduced FVIII activity (FVIII:C) levels as a result of skewed, random X chromosome inactivation (referred to as lyonization)[25]. These girls and women are now diagnosed as having haemophilia, with FVIII levels comparable to those of men with non-severe haemophilia A[26], and are at additional risk of more severe symptoms as a result of gynaecological issues, for example, excessive menstrual bleeding. Homozygosity, compound heterozygosity or XO karyotype (known as Turner syndrome, in which one of the X chromosomes is missing or partially missing) with a defective *F8* or *F9* gene (or both) are rare scenarios of potentially severe haemophilia in a woman or girl.

The *F8* mutations giving rise to haemophilia A are numerous but largely categorized, from most to least disruptive, as large deletions, nonsense mutations, inversion of intron 22 or intron 1 and missense mutations[27]. Inversion of intron 22 is the most common causative defect for severe haemophilia A, arising in approximately 40% of cases[28]. Inversion events are thought to happen most commonly during male meiosis. The size-imbalanced, pseudo-autosomal XY pairing permits movement at the tip of the long arm of the longer

X chromosome back on itself, with consequent inverted, non-functional, intrachromosomal homologous recombination. For a boy living with severe haemophilia A due to inversion of intron 22 without a family history of the disease, this inversion event might have occurred generations earlier in the spermatogenesis of his maternal grandfather. Point mutation, missense *F8* genotypes are responsible for the majority of cases of non-severe haemophilia A; *F8* variants are available at the European Association for Haemophilia and Allied Disorders (EAHAD) variant database.

Individuals with haemophilia A are treated with replacement factor therapy, consisting of intravenous therapeutic FVIII (tFVIII, see Management section), either as prophylaxis or episodically to treat bleeding events, trauma or surgical procedures. Of note, an immune response against tFVIII can seriously complicate replacement factor treatment. tFVIII-derived peptides are presented via class II MHC proteins on the surface of antigen-presenting cells, priming a helper T (T_H) cell population, which in turn drives an oligoclonal expansion of responsive, antibody-producing B cells. The resultant anti-tFVIII allo-antibodies recognize the infused tFVIII as a foreign antigen and, by binding to tFVIII, inhibit the function of the infused factor; thus, these antibodies are known as inhibitors[29,30] (see Management section). It is not known why only a proportion of individuals experience a clinically relevant inhibitory response. The *F8* genotypic defect is a major contributor to the risk of developing an inhibitor, in the context of tFVIII exposure intensity and background of other genetic polymorphisms of MHC and immunomodulatory pathways (for example, single nucleotide polymorphisms in *TNF* or *CTLA4*)[31–34]. It is not known how each immune response polymorphism individually contributes to the risk of developing anti-tFVIII antibodies; instead these polymorphisms are additional, potential biomarkers of risk. Inhibitor risk in people carrying the more disruptive *F8* genotypes causing severe haemophilia A is up to 40%, occurring predominantly during the first 50 days of tFVIII exposure and usually early in life[30]. However, people with non-severe haemophilia A caused by a single point mutation may still have lifetime inhibitor risk in excess of 10%, and, for some patients (for example, those with the W2229C mutation), close to that associated with severe haemophilia A[35].

FVIII. FVIII is a large, heterodimeric plasma protein, consisting of heavy (A1-A2 domains) and light (A3-C1-C2 domains) chains. It has species-specific and tissue-specific glycosylation and sulfation patterns[36,37], the relevance of which continue to be explored in human and non-human recombinant cell lineages used in concentrate manufacture. The in vivo cell lineage that produces and releases functional FVIII protein has recently been identified to be endothelial cells rather than hepatocytes[38–40]. FVIII circulates in an inactive form bound to the D′D3 domain of the chaperone protein vWF and as such is confined to the intravascular compartment. vWF itself is also a large, multi-domain clotting plasma protein that is pivotal to platelet adhesion and localization of primary haemostasis.

Upon thrombin activation, FVIII undergoes proteolytic cleavage at positions pArg372, pArg740 and pArg1689 (REF.[41]). Cleavage at pArg372 and pArg1689 positions is essential for full FVIII activation (FVIIIa). Cleavage exposes a functional site within the A2 domain that is required for interaction with FIXa, which is hidden in the inactive form of FVIII; FVIIIa uncouples from vWF, with a subsequent conformational change further enhancing its activity[42]. FVIIIa then binds to the negatively charged phospholipids on the surface of the platelet plug via its light chain C1-C2 domains[43]. The presence of early fibrin production enhances this binding[44]. Platelet-bound FVIIIa provides the anchor for an enzymatic complex (the tenase complex) consisting of FIXa (the enzyme) and factor X (the substrate). The resultant activated factor X (FXa) then joins an analogous enzymatic complex (the prothrombinase complex) with a FVa anchor, binding FII (also known as prothrombin, the substrate) to produce thrombin[45,46]. Both FIX and FX also have phospholipid-binding capability via calcium-dependent γ-carboxyglutamate (Gla) domains[47]. With increased phospholipid binding avidity, the bound FVIIIa optimizes the co-localization of FIXa and FX, amplifying the activation of FX up to 10^6-fold[48]. Thus, partial or total deficiency of FVIIIa, or indeed FIXa, is the basis of an inadequate thrombin burst in haemophilia A and B, respectively. The greater the deficiency, as determined by how disruptive the *F8* mutation is, the greater the likelihood of spontaneous bleeds, in addition to the inevitable excessive bleeding due to minor trauma or surgery in the absence of therapeutic replacement with exogenous clotting factor concentrate (tFVIII). The ability of a plasma sample to generate thrombin (and thereby convert fibrinogen into fibrin) or FXa in vitro underpins routine diagnostic and factor replacement surveillance clotting assays (see Laboratory tests section below).

Haemophilia B

F9. Cloning of *F9* in 1982 (REF.[49]) facilitated the mutational description of haemophilia B and highlighted the importance of genetic counselling. Haemophilia B is genetically heterogeneous, with a predominance of missense mutations, without a common inversion event analogous to the inversion of intron 22 in *F8*, in the absence of any additional homologous sequences with which *F9* could recombine. *F9* variants are available at the EAHAD variant database. Protein-truncating mutations (deletions and nonsense mutations) are less common than in *F8*, explaining, in part, the different incidence of inhibitor occurrence: <10% incidence in haemophilia B in the first 75 exposures to tFIX compared with >30% risk in haemophilia A[50]. Inhibitors in non-severe haemophilia B are almost non-existent; this phenomenon is poorly studied in haemophilia B, owing to the rarity of the disease and consequent lack of large cohort genetic studies analogous to those performed in haemophilia A[35].

Haemophilia B Leyden is a subgroup of haemophilia B that is characterized by low levels of FIX in the early years of life, which then rise and potentially normalize into adulthood. This subgroup is attributable to

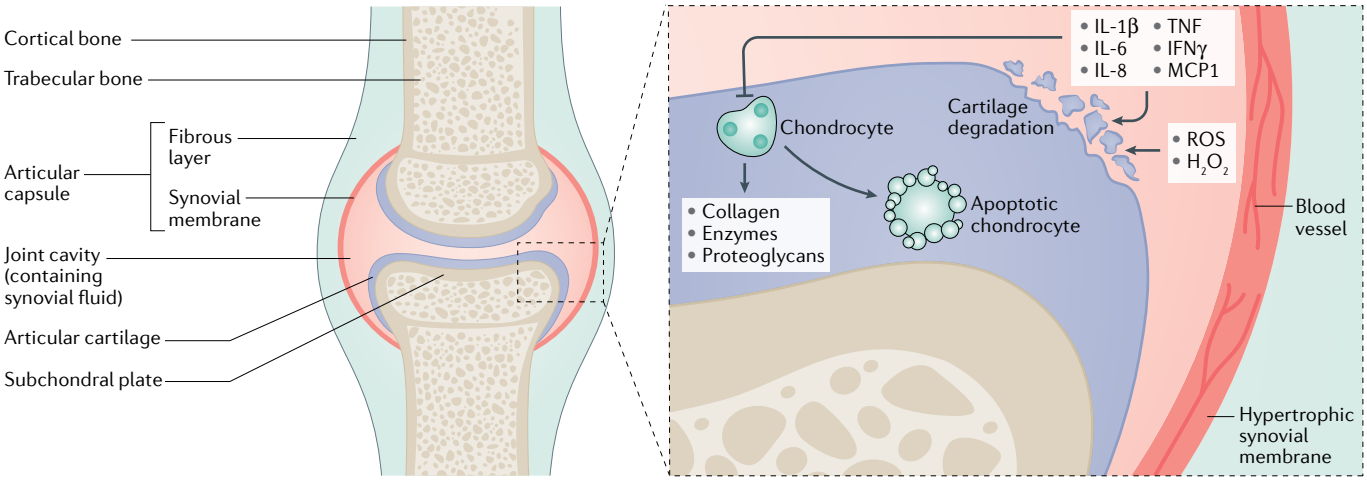

Fig. 2 | **Pathophysiology of haemophilic arthropathy.** Articular cartilage consists of chondrocytes embedded in an extracellular matrix. Chondrocytes produce collagen, proteoglycans and enzymes that are responsible for cartilage metabolism and health[64]. The synovial membrane lines the inner surface of capsules of synovial joints and is normally composed of two to three cell layers with small blood vessels that provide nutrients to the synovium and joint, including the articular cartilage[64]. H_2O_2, hydrogen peroxide; IFNγ, interferon-γ; MCP1, monocyte chemoattractant protein 1; ROS, reactive oxygen species; TNF, tumour necrosis factor. Adapted from REF.[227], Springer Nature Limited.

promoter mutations that compromise transcription in the pre-pubertal period but are overcome by an androgen response element in the promoter that is responsive to the androgen burst of puberty[51,52].

Finally, a gain-of-function FIX variant identified as a cause of a rare X-linked thrombophilia (an excessive clotting risk), the Padua variant (R338L), has since been used successfully to optimize haemophilia B gene therapy strategies[53]. The hyperactivity of this variant depends on enhanced interaction with the FVIIIa cofactor in the tenase complex, leading to an eightfold increase in activity[54].

FIX. In contrast to FVIII, FIX is synthesized in hepatocytes, is vitamin-K-dependent for enzymatic carboxylation of the glutamic acid residues of the phospholipid-binding Gla domain, and is a substantially smaller protein (415 amino acids compared with 2,332) without requirement for a chaperone. Owing to its ability to move into the extravascular space passively because of its molecular size, FIX is distributed extravascularly, some binding to subendothelial collagen[55].

As the key enzymatic component of the tenase complex, FIX requires an activation step. This activation is a combination of FVIIa-dependent activation during haemostasis initiation[56] and thrombin-activated FXI (FXIa)[57,58] (FIG. 1b); carboxylation of the glutamic acid residues prompts a conformational optimization of the Gla domain after exchange of Mg^{2+} for Ca^{2+} at a central binding pocket[59], and this conformational change enables phospholipid binding by the Gla domain. FIXa binds to the tenase cofactor, FVIIIa, enabling hydrolysation of FX to the active form FXa. FXa is the subsequent enzymatic contributor to the downstream prothrombinase complex, generating thrombin. Suboptimal burst of thrombin activity in haemophilia B is equivalent to that underpinning haemophilia A pathophysiology (FIG. 1c).

Pathophysiology of haemophilic arthropathy

The pathophysiology of haemophilic arthropathy is multifactorial and seems to be induced directly by the interaction of blood with articular cartilage and indirectly by inflammation[60–62]. It is well established that recurrent bleeding into synovial joints leads to severe joint destruction, partly resembling the degenerative articular cartilage damage seen in osteoarthritis, as well as the synovial and inflammatory processes of rheumatoid arthritis[60,63–65]. Bleeding is most common in the large synovial joints, predominantly the ankle, knee and elbow joints, as they are primarily single planar joints, in contrast to the hip and shoulder, which are multi-planar[66,67]. Additionally, low expression of tissue factor in normal joint and muscle tissue affects clot formation and contributes to the predisposition of bleeding in these sites[68]. As weight-bearing loading joints are responsible for force transfer, the knee and ankle are more commonly affected than the elbow, which is usually secondarily affected owing to increased use of the upper limb (for example, use of walking aides or while sitting and standing up) as a result of lower limb arthropathy. As bleeding events have become less frequent owing to improved therapy, the ankle joint seems to be more affected than the knee, possibly owing to its thinner layer of articular cartilage.

In a haemophilic joint exposed to repeated bleeding, the synovium becomes hypertrophied and protrudes into the joint space[69] (FIG. 2). Histologically, haemosiderin deposition is observed, which has been shown in animal and human models to play a part in further synovial proliferation[70]. Investigating the early changes induced by haemarthrosis is challenging, because arthropathy is usually asymptomatic in the early stage. Human in vitro models have demonstrated chondrocyte apoptosis and reduced proteoglycan synthesis that affects cartilage matrix turnover within 48–96 h of an induced joint bleed, suggesting that a single joint bleed may have detrimental effects on joint cartilage[60,70–73].

Studies suggest that the pathophysiology of haemophilic arthropathy is multifactorial and is induced indirectly by inflammation and directly by the interaction of blood with articular cartilage, with iron and inflammatory cytokines IL-1β and tumour necrosis factor (TNF) central to the process. Excessive exposure of synovial tissue to iron released from erythrocytes leads to synovial proliferation and macrophage production of cytokines such as IL-1β, TNF, interferon-γ (IFNγ), monocyte chemoattractant protein 1 (MCP1; also known as CCL2), IL-6 and IL-8 that inhibit chondrocyte activity leading to cartilage destruction[61,71,73–75]. It has been proposed that IL-1β and TNF induce production of pro-inflammatory cytokines such as IL-8 and IL-6 (REF.[61]), and this production is enhanced by the presence of haemosiderin-laden synovium[76]. Although synovium is involved, in vitro studies have shown that erythrocyte-derived iron and IL-1β-induced hydrogen peroxide contributed to the direct destruction of the cartilage matrix, by inducing the formation of reactive oxygen species and reacting with haemoglobin-derived iron to form hydroxyl radicals, respectively[77,78]. The direct effects on cartilage seem to be exacerbated by weight bearing[62] and precede the indirect inflammation-related effects[78]. However, the in vivo role of iron and pro-inflammatory and anti-inflammatory cytokines and signalling pathways requires further investigation.

Diagnosis, screening and prevention

Haemophilia is a genetic disease, hence the identification of the causative genetic mutation in affected individuals is important for familial counselling and has a predictive value for bleeding tendency and inhibitor risk[79–81]. Although the severity of haemophilia is defined by overall bleeding manifestations, the individual clinical phenotype varies within each group. In general, patients with severe haemophilia are diagnosed before the age of 2 years, but individuals with mild haemophilia may remain undiagnosed for several years, as some only manifest bleeding symptoms at the time of injuries or associated with surgery[82]. On average, 25–50 bleeding events per year could occur in individuals with severe disease, 5–10 in those with moderate disease and 1–2 in those with mild disease, if untreated, but nowadays prophylaxis is the recommended standard of care worldwide[23,83]. The infrequency of bleeding episodes in patients with mild factor deficiency often leads to treatment delay, resulting in prolonged therapy or additional complications. The laboratory phenotype is related to the bleeding phenotype (that is, the bleeding tendency), and the more severe the deficiency of the specific clotting factor activity, the younger the age at diagnosis when based on clinical symptoms. The median age at diagnosis of severe haemophilia is between 12 and 18 months; mild haemophilia can be diagnosed even during adulthood if a positive family history is unknown or not present[23].

Diagnosis

There are three main clinical scenarios that can lead to haemophilia diagnosis: a positive family history of the disease, a history of abnormal bleeding in the individual or close family members, or the unexpected finding of abnormal coagulation tests.

Positive family history. When there are one or more known individuals with haemophilia in a family, it is possible to ascertain the carrier status of women and girls and to make an early diagnosis in potentially affected men and boys. Owing to the X-linked genetic transmission of the disease and in combination with a detailed family history, it is possible to identify obligate female carriers and possible female carriers. Obligate carriers are: daughters whose father has haemophilia, mothers of more than one haemophilic child and mothers of one haemophilic child with another relative with haemophilia on their mother's side (for example, a brother or a maternal uncle). Of note, having only one child with haemophilia is not sufficient to determine that the mother is an obligate carrier, as haemophilia in the child could be due to a de novo mutation. Possible carriers are all women with at least one haemophilic male in their maternal pedigree. Normal FVIII and FIX plasma levels cannot rule out the carrier status of a female, because they are reduced only in approximately 30% of cases[79,80]. The only way to ascertain the carrier status is through molecular diagnosis of the causative mutation in *F8* or *F9* (REF.[81]). This type of diagnosis is much easier if the mutation is already known in the index case (that is, the patient with haemophilia).

Bleeding tendency. Neonates with haemophilia are rarely affected by severe bleeds; nevertheless, cerebral haemorrhage is possible at a higher incidence than in the general population (3–10% in children with haemophilia, whereas a 20- to 50-fold lower incidence has been reported in unaffected children)[84], and it is mainly related to instrumental vaginal delivery and/or prolonged labour, which should be avoided in known or possible carriers[85]. Cerebral haemorrhage in a neonate should indicate suspected haemophilia and prompt further investigations.

The presence of unusual bleeding symptoms in young male children may suggest haemophilia. Typically, children with severe or moderate haemophilia show easy bruising around 8–10 months of age, and this finding may prompt coagulation tests to ascertain the specific deficiency. During the first year of life, and especially when crawling and/or beginning to walk, muscle bleeds (for example, gluteal haematoma) and/or joint bleeds may also occur; these bleeding events represent the most common bleeding symptoms of untreated haemophilia throughout life[23]. Children with mild haemophilia may not show any bleeding symptoms until adulthood if they do not incur severe trauma or surgery, which in turn represent the main haemostatic challenges in this group of patients.

Accidental finding of abnormal coagulation tests. Diagnosis of haemophilia can be made during investigation of incidental findings of abnormal coagulation tests. Isolated prolongation of the activated partial thromboplastin time (aPTT; that is, one of the tests that are commonly done to screen coagulation activity) can

be found in patients undergoing laboratory examination before surgery and might be due to a moderate or mild form of the disease, which can be diagnosed in adulthood. Laboratory confirmation of haemophilia includes a mixing test with normal plasma to rule out possible inhibitory activity from antibodies, as in the case of lupus anticoagulants (that is, polyclonal antibodies usually directed against phospholipids), and then specific measurements of the clotting factors involved in the intrinsic pathway of coagulation (that is, FXII, FXI, FIX and FVIII) to ascertain specific deficiencies.

In all cases diagnosed because of abnormal bleeding and/or the unexpected finding of abnormal tests, a careful family history should be collected to rule out the presence of other possible affected men and boys and to perform a proper genetic counselling to all possible carriers.

Laboratory tests

Whichever is the scenario that leads to the suspicion of haemophilia, the definite diagnosis is made by measurement of residual FVIII and FIX clotting activity (FVIII:C and FIX:C). These measurements can be performed using either one-stage or chromogenic clotting assays. The one-stage assay is based on the aPTT test, and the calculation of residual levels of FVIII or FIX is done by comparing aPTT measured in the test plasma (mixed with either FVIII-deficient or FIX-deficient plasma) with that measured in serial dilutions of standard reference plasma (that is, with given FVIII or FIX concentration)[86]. The chromogenic assay is based on the measurement of the amount of FXa generated in plasma, which is proportional to the level of functional FVIII or FIX. In this assay, the concentration of FXa is quantified using a chromogenic substrate specific for FXa, with the intensity of the colour generated being proportional to the level of FXa generated[86]. Chromogenic assay does not require the addition of factor-deficient plasma and is less prone to interference by some pre-analytical variables (for example, the time elapsed between blood sampling and laboratory testing) than the one-stage assay is. The one-stage assay is the most commonly used because of its long-standing use and because it is perceived to be cheaper than the chromogenic assay. However, there is evidence of comparable costs for the two assays if an efficient use of reagents is pursued[87]. The one-stage assay is generally used for diagnosis of haemophilia; however, in some specific situations both assays should be used. In fact, the chromogenic assay is useful for the accurate diagnosis of non-severe haemophilia A, because in some cases assay discrepancies have been reported[88] depending on the specific *F8* mutation[26,89,90]. In mild haemophilia A, a ratio of >2.0 or <0.5 between the severity measured with the one-stage assay and that measured with the chromogenic assay indicates a substantial discrepancy. In most cases of discrepancy, both assays are <40 IU/dl, but there are cases in which the results of one of the assays are within the normal range, and the diagnosis would be missed if only one test were performed.

Genetic analysis to identify the causative mutation of the disease is routinely carried out for severe and moderate haemophilia but less frequently in mild haemophilia for various reasons, including that prenatal diagnosis is less frequently required in the case of mild disease, the cost of testing can be an issue and the prognostic implications of the genetic profile for patients with mild disease are much less relevant than for those with severe disease. Genetic analysis is usually carried out when a diagnosis in an individual with no family history is made: for haemophilia B, whole-gene sequencing of *F9* is usually performed, whereas for haemophilia A the first step is to look for intron 22 and intron 1 inversions in *F8*, which account for approximately 40% and 1% of severe cases, respectively, followed by whole-gene sequencing.

Prevention

As haemophilia is a rare X-linked disease, screening with FVIII:C or FIX:C measurement is recommended in male relatives of the mother of a new index case, and genetic screening is recommended to identify female carriers. Education of female carriers is important for disease prevention; genetic testing is not mandatory for women or girls who are obligate carriers, whereas it is the only way to identify carriers in all other scenarios. Female carriers should be educated on the probability of having a haemophilic son and on all the existing procedures that could prevent this occurrence.

Knowing the mutation in the family also enables the implementation of prenatal diagnosis, which can be done at different stages: on the germinal line of the female carrier for pre-implantation genetic diagnosis (PGD)[91], on chorionic villi during the first trimester of pregnancy[92] or on amniocytes during later stages of pregnancy[93].

PGD offers the possibility to select unaffected embryos but implies that the pregnancy occurs through in vitro fertilization procedures. Chorionic villus sampling (CVS) performed between the 11th and 14th weeks of gestation enables early genetic diagnosis with subsequent possibility of termination of pregnancy; in some cases, pregnant women who are carriers undergo CVS because they want to know beforehand whether or not a male fetus is affected with haemophilia. In this scenario, mothers should be informed about the risk (although very low; around 1%) of procedure-related miscarriage[94]. Genetic diagnosis through amniocentesis can be used to ascertain the presence of the disease in a male fetus, but it is performed at a gestational age that might be beyond the limit for voluntary termination.

Recently, non-invasive techniques have been introduced and optimized to determine fetal sex by the analysis of cell-free fetal DNA in maternal blood through peripheral blood sampling from the seventh week of gestation[95]. These tests may reduce the number of women undergoing invasive procedures when pregnancy termination is not their option, but it does not yet provide a definitive diagnosis.

If prenatal diagnosis is not performed, it is possible to make diagnosis of haemophilia in a newborn boy from a female carrier at birth by measuring FVIII:C or FIX:C levels in cord blood. This measurement is highly reliable for FVIII, whereas in the case of moderate or

mild haemophilia B the measured level of FIX:C can be lower than expected owing to partial liver immaturity and physiological vitamin K deficiency of newborn infants. In those cases, it is recommended to repeat the measurement at 9–12 months of age.

Approximately 30% of individuals newly diagnosed with haemophilia have no family history of disease and the mother is not a carrier[96]. In these cases, the disease can be attributed to a de novo mutation in *F8* or *F9* (REFS[96,97]). A possible alternative explanation for affected individuals with a non-carrier mother is the presence of a germline mosaicism in the mother[98]. In this case, the mutation is present only in some of the germline cells and, therefore, it cannot be detected in peripheral white blood cells, which are the cells typically used to perform genetic analysis. Because the fact that a mutation could not be identified from peripheral white blood cells does not rule out the possibility of germline mosaicism (and, therefore, the possibility that haemophilia in the first son is not caused by a de novo mutation), non-carrier mothers who already have a son with haemophilia should be informed of the possibility to perform prenatal diagnosis in the case of a second pregnancy with a male fetus.

Management

Effective management of haemophilia is essential to avoid unnecessary bleeding and long-term sequelae; prophylactic therapies to reduce the frequency of bleeding and on-demand regimens to treat bleeding as it occurs are considered the mainstay of haemophilia treatment worldwide[99–101]. Persons with haemophilia should have access to comprehensive care facilities, with a multidisciplinary specialist team (including at least physicians with expertise in bleeding disorders, nurses, physical therapists and/or musculoskeletal specialists, and social workers and/or occupational therapists) and 24 h access to a specialized coagulation laboratory, to provide coordinated, ongoing supervision for all medical and psychosocial needs of patients and their families[102,103]. Access to comprehensive care significantly reduces mortality (by 40%) and hospitalization rates[104,105]. As patients may now achieve a normal lifespan, common ageing issues and their manifestation in individuals with haemophilia must be addressed[106–109]. Important issues related to transition from paediatric to adult haematology care providers is also best assured through comprehensive care and centres.

Box 1 | **Current definitions of on-demand and prophylaxis therapies**

- On-demand therapy: replacement factor given at the time of bleeding
- Continuous prophylaxis: replacement factor given to prevent bleeding for at least 45 of 52 weeks (85%) of a year
  - Primary prophylaxis: continuous prophylaxis started before 3 years of age and before the first or second large joint bleed
  - Secondary prophylaxis: continuous prophylaxis started after two or more large joint bleeds but before the onset of chronic arthropathy
  - Tertiary prophylaxis: continuous prophylaxis started at the onset of arthropathy to prevent further damage
- Intermittent prophylaxis: replacement factor given to prevent bleeding for short periods of time such as during and after surgery

Treatment guidelines

The mainstay of haemophilia therapy is replacement of the deficient clotting factor to achieve adequate levels for cessation or prevention of bleeding. Wherever possible, only the specific deficient factor should be replaced[110], to avoid administration of unnecessary proteins, including agents that may have unwanted safety concerns or thrombotic potential, such as prothrombin complex concentrates in FIX deficiency. Two primary approaches exist for replacement therapy for treatment of haemophilia: prophylaxis, to prevent bleeding events, and on-demand therapy, to treat haemorrhage when it occurs (BOX 1). Intermittent prophylaxis is typically used around surgery for patients who are not on continuous prophylaxis and for some patients participating in sports; these days the choice between intermittent and continuous prophylaxis is probably mostly a cost issue. Regimen choice is dependent upon the level of clotting factor deficiency, bleeding phenotype, availability of resources and the preference of patients or their families.

Prophylaxis. Although primary prophylaxis (BOX 1) is the preferred therapeutic option[111,112], its cost may limit use in resource-constrained countries, where either on-demand therapy or limited prophylaxis with reduced doses or longer intervals between infusions might be used[110]. Where prophylaxis is routinely used, there may be wide variations in standard practice. A significant correlation between the rate of breakthrough joint bleeding (that is, spontaneous bleeding occurring during prophylaxis) and time during which FVIII levels remain <1 IU/dl was demonstrated[113]. In young patients (1–6 years of age), a 10% increase in the time during which FVIII levels were <1 IU/dl (17 h per week) correlated with a 44% increase in bleeding events per year; conversely, if minimal levels were always maintained at >1 IU/dl, there would be one fewer bleeding per year predicted to occur. Similar trends were observed in older patients. No comparable data are available for haemophilia B.

Traditionally, prophylactic therapy targets a minimal trough of 1–3 IU/dl of the deficient factor, with the optimal trough level adjusted depending on the individual's breakthrough bleeding rate[83]. Nevertheless, higher trough levels are preferred to suppress all breakthrough bleeding (including subclinical bleeds), prevent long-term disease sequelae (for example, chronic arthropathy) and achieve a normal lifestyle[114]. This observation is based on the comparison of long-term outcomes in patients treated with intermediate-dose prophylaxis in the Netherlands and high-dose prophylaxis in Sweden; those on the high-dose regimen experienced fewer bleeds and improved joint scores than those on the intermediate-dose regimen[115]. Similarly, in a 26-year longitudinal study, even with long-term prophylaxis, joint disease remained common in persons with haemophilia[116,117]. Trough factor levels would have to exceed 15 IU/dl to achieve zero bleeds, a level that is essentially unattainable with current replacement therapy, owing to product requirements and costs[118,119]. The existence of joint damage in patients reporting zero or very few bleeds has been attributed to the presence of subclinical bleeding[111,120]. Thus, early diagnosis of

Table 1 | **Common sites of bleeding in haemophilia and WFH recommended FVIII replacement therapy**[a]

Site of bleeding	Typical target FVIII range (IU/dl)	Typical duration of replacement therapy (days since bleed or surgery)
Joint (60% of bleeding episodes)	40–60	1–2
Muscle (30% of bleeding episodes)	40–60	2–3
GI tract	80–100 (initial)	7–14
	50 (maintenance)	
Iliopsoas	80–100 (initial)	1–2
	30–60 (maintenance)	3–5
Throat and neck	80–100 (initial)	1–7
	50 (maintenance)	8–14
Renal	50	3–5
CNS	80–100 (initial)	1–7
	50 (maintenance)	8–21
Deep laceration	50	5–7
Surgery (major)	50–80 (pre-op)	Pre-op
	60–80 (post-op)	1–3
	40–60 (post-op)	4–6
	30–50 (post-op)	7–14
Surgery (minor)	50–80 (pre-op)	Pre-op
	30–80 (post-op)	1–5

CNS, central nervous system; FVIII, factor VIII; GI, gastrointestinal; IU, international units; WFH, World Federation of Hemophilia. [a]WFH recommended FVIII replacement therapy in countries with no substantial resource constraints[83].

synovitis is important, and ultrasonography may have a role in regular routine monitoring of potential subclinical bleeding[121].

On-demand therapy. Although prophylaxis reduces the risk of breakthrough bleeds, it may not eliminate them[114], and thus, there is a requirement for access to additional doses for prompt treatment[122]. The World Federation of Hemophilia (WFH) has published recommendations for the treatment of common bleeds[83] (TABLE 1); however, these are minimal recommendations, and treatment must be tailored to achieve individual optimal outcomes on the basis of the site and extent of bleeding, individual response and resolution. Bleeding at different sites requires different dosing of replacement therapy, on the bases of the likelihood of damage and the length of time required to resolve (for example, intracranial haemorrhage or bleeding of other vital organs would be treated aggressively to reduce the risk of long-term sequelae). Prompt treatment for all bleeding episodes is essential, both to treat immediate symptoms including pain and swelling and to limit longer-term sequelae; thus, home-based treatment represents ideal care[105].

Biomarkers

Factor-specific assays are used to track factor levels as clinical biomarkers for treatment efficacy and are more precise than global haemostatic assays, such as a thrombin generation assay. Reagent choice and assay (one-stage, two-stage or chromogenic) may affect the accuracy of the measurements of specific factors; attention to the manufacturer's recommendations for each factor concentrate is crucial[123]. For patients on prophylaxis, the annualized bleeding rate (ABR) is a surrogate clinical biomarker; optimal prophylaxis ABRs should trend towards zero. Identification of serological biomarkers of ongoing joint damage in the serum or urine of patients represents an additional area of active research; such biomarkers may ultimately complement factor assays and imaging modalities in assessing disease progression in individuals with haemophilia[124].

Products and therapies

Replacement therapy. Although cryoprecipitate and fresh frozen plasma (FFP) are routinely used in some countries (possibly owing to lower cost than specific factor concentrates), they are not consistently virally inactivated to avoid transmission of blood-borne pathogens, and FFP may not adequately ensure haemostasis; thus, they are not optimal options[110]. The development of extended half-life (EHL) factor concentrates has steadily advanced over the past several years. Standard half-life (SHL) FVIII products have half-lives of 10–12 h (REF.[125]); the half-life of EHL FVIII concentrate products is approximately 1.5 times longer than that of SHL products, which translates into minimal twice-weekly prophylactic administration[125]. Several bioengineering strategies exist to prolong the half-life of FVIII concentrate. FVIII fusion to albumin or antibody fragment crystallizable (Fc) domains enables avoidance of endolysosomal degradation clearance mechanisms, whereas PEGylation, that is, conjugation to polyethylene glycol (PEG), is thought to reduce FVIII susceptibility to proteolysis and renal clearance. Finally, with single-chain technology, the FVIII heavy and light chains are covalently bound together to form a novel recombinant protein with greater stability, improved vWF affinity and longer half-life[125,126]. The increase in FVIII half-life is ultimately limited by the half-life of its carrier protein, vWF ($t_{1/2}$ = 16 h) (see Pathophysiology section).

SHL FIX products have half-lives of 18–20 h (REF.[125]). The effects of PEGylation and fusion protein modification on the half-life of EHL FIX products are more pronounced than current FVIII EHL products (twofold to fivefold increase, as the half-life of FIX is not limited by that of a carrier protein)[126], extending administration intervals up to approximately every other week depending on the desired target trough[125]. Dosing regimens are tailored and adjusted on an individual basis, guided by pharmacokinetic profile, physical activity (for example, dose administration may be adjusted to immediately precede strenuous or increased-risk physical activity to reduce the risk of bleeding), and clinical observation. Bayesian statistical models enable estimation of product-specific pharmacokinetic curves using limited time-point analysis, reducing patient inconvenience[127].

Inhibitor development and treatment. The development of inhibitors (that is, the emergence of neutralizing antibodies to exogenous FVIII or FIX) is one of

the most serious complications of replacement therapy and occurs in approximately 30% of individuals with severe FVIII deficiency and approximately 10% of those with severe FIX deficiency[50,128]. Inhibitor formation rate varies on the basis of various factors, including level of deficiency and race/ethnicity (for example, individuals with African or Hispanic backgrounds have been shown to have an increased risk of inhibitor formation)[129]. Inhibitor development may be associated with some genetic (for example, complete deletion of *F8* or *F9*) and environmental factors, yet all contributing influences have not been completely elucidated. The class of the replacement product has also been implicated: the SIPPET study suggested an increased rate of inhibitor development with recombinant product compared with plasma-derived products[30]. Following this observation, national guidelines were updated, reinforcing the view that inhibitor formation is probably patient and product (rather than product class) specific, and that changes in current therapies are not warranted without further investigation[130].

Inhibitor treatment options vary depending on titre, commonly measured with the Nijmegen modification of Bethesda assay (NBA); of note, nonspecific coagulation inhibitors (for example, lupus anticoagulants) can interfere in the assay, causing false positives[131]. Inhibitors most commonly emerge within the first 50 days of exposure, although they may occur at any point during a patient's lifetime[132,133]. Patients with mild haemophilia A may develop inhibitors later in life or after severe trauma or surgery requiring intensive treatment[134]. In these patients, inhibitors may recognize either both exogenous and endogenous FVIII, with a resultant more severe bleeding phenotype, or only the exogenously administered product[135]. In this circumstance, normal baseline levels of FVIII remain, yet the patient may not respond to standard therapy, as the exogenously administered product would be neutralized by the inhibitors. All patients with haemophilia require ongoing surveillance for inhibitors, ideally at least once yearly, or more frequently as indicated[136].

Patients with inhibitor titres of approximately 0.5–5 Nijmegen Bethesda Units (NBU) despite repeated exposures are termed 'low responders' (of note, the lower limit of detection for NBAs varies slightly by test manufacturer and analysing laboratory). Low responders may be treated with an increased factor concentrate dose that is sufficient to achieve haemostasis (the normal dose required for haemostasis plus a 50% correction per NBU). However, such a strategy is not feasible for patients with inhibitor titres >5 NBU (termed 'high responders'); instead, bypassing agents (BPAs) are employed to achieve haemostasis. Commonly used BPAs include recombinant factor VIIa (rFVIIa) and activated prothrombin complex concentrates (aPCCs), used either on-demand or as prophylaxis[137,138]. A new rFVIIa variant that offers different dosing regimens and with high reported efficacy has been approved for use by the FDA[139]. A plasma-derived FVIIa:FX concentrate (1:10 ratio) is available in Japan for the treatment of bleeding events in patients with inhibitors. Its increased efficacy over rFVIIa has been attributed to the preferential formation of the FVIIa–FX–TF complex over the competing FVIIa–FIX–TF complex[140].

Variability in inter-individual and intra-individual response to BPAs has been documented and, therefore, close clinical supervision by knowledgeable health-care providers is necessary[141]; lack of response is not predictable. Furthermore, there are no validated biomarkers for BPAs that correlate with clinical efficacy; effectiveness is assessed by clinical response. Patients with FIX inhibitors may experience anaphylactoid reactions to FIX-containing products, necessitating either desensitization or use of rFVIIa[110]. Such reactions have only been rarely reported in haemophilia A. In low responders, inhibitors may spontaneously resolve with time[142], whereas in high responders they rarely resolve without specific therapy. Both low responders and high responders may undergo immune tolerance induction (ITI) for inhibitor ablation. ITI involves frequent doses of FVIII or FIX over time to induce tolerance and can be intensive, lengthy and costly[143,144]. ITI treatment guidelines are available[145,146]; recent data support early ITI regardless of inhibitor titre, instead of waiting until the titre is <10 NBU[147]. Breakthrough bleeds on ITI are treated with BPAs. ITI in patients with mild haemophilia A, as well as in haemophilia B, is more successful when using immunomodulatory agents in addition to FVIII or FIX, respectively, than when replacement factors are used alone. Patients with haemophilia B with inhibitors may require desensitization (induction of drug tolerance) before ITI initiation, and may experience re-emergence of anaphylactoid reactions and development of nephrotic syndrome while on ITI[148]. In such patients, the use of additional immunosuppressant therapies has been employed with some success[149].

Non-replacement products and novel agents. Several non-replacement agents are currently being investigated[150], including fitusiran[151], Super FVa[152], factor Xa[153], APC inhibitors[154] and TFPI inhibitors[155] (TABLE 2). In mild FVIII deficiency, desmopressin acetate administered intranasally, subcutaneously or intravenously increases FVIII levels, and is commonly used as first-line treatment in patients who respond[83]. Desmopressin causes the release of FVIII, vWF and plasminogen activator from endothelial cell storage sites, and it is much less costly than FVIII concentrates. A test dose to document an adequate haemostatic level should be performed. Desmopressin is not effective in haemophilia B.

A novel, non-replacement agent, emicizumab, is licensed for routine, subcutaneous prophylaxis to prevent or reduce the frequency of bleeding episodes in persons with haemophilia A[156,157]. Emicizumab is a biphenotypic antibody with specificity for both FIX and FX and mimics the role of FVIII in the tenase complex, localizing FIXa close enough to FX to enable activation and downstream contribution to the coagulation cascade. Of note, emicizumab cannot be used in persons with haemophilia B[8,158]. Emicizumab cannot be used to treat breakthrough bleeding, as it is already present at a steady state level; breakthrough bleeding requires administration of FVIII in patients without inhibitors and BPAs in patients with inhibitors.

Table 2 | **Non-replacement investigational treatments for haemophilia currently in clinical development**

Product	Phase	Treatment for	Comments
Fitusiran	III	HA and HB	RNA interference agent that targets antithrombin[222]. The study was placed on hold following a fatal thrombotic event; hold lifted in December 2017 after risk mitigation strategies were developed. The study was put on hold again in October 2020 and resumed in December 2020
Super FVa	Preclinical	To be determined	FVa variant, resistant to APC cleavage. Demonstrated synergistic activity with rFVIIa in animal models
FXa	I	HA and HABI	FXa variant with increased resistance to inactivation by plasma proteases. Phase I in healthy volunteers completed. Safety and tolerability study for the treatment of intracerebral haemorrhage was terminated in October 2017 for strategic, and not safety, reasons[223]
APC inhibitors	I/II	HA, HB and HABI	Inhibitors are being developed to block the natural anticoagulant mechanism of APC. A phase I study for the lead candidate, KRK-α_1AT (α1-antitrypsin with three residue changes), is ongoing[224]
TFPI inhibitors	III	HA, HB and HABI	Concizumab and marstacimab, monoclonal antibodies that target TFPI, are currently in clinical development[225]. The development of BAY-1093887 was discontinued in 2019 owing to thrombosis[226]. Three episodes of thrombosis have also been reported with concizumab, which led to a pause of all concizumab clinical trials in March 2020; subsequent risk mitigation strategies enabled the trial to be resumed in August 2020

APC, activated protein C; FVa, activated factor V; FXa, activated factor X; HA, haemophilia A; HB, haemophilia B; HABI, haemophilia A or B with inhibitors; rFVIIa, recombinant activated factor VII; TFPI, tissue factor pathway inhibitor.

Emicizumab has demonstrated efficacy for effective prophylaxis in patients with haemophilia A with inhibitors: clinical studies showed a significant reduction in ABR compared with either on-demand or prophylactic BPA regimens[159]. Emicizumab is administered every 7, 14 or 28 days as a subcutaneous injection. Breakthrough bleeding events still occur with prophylactic emicizumab, although the bleed rate decreases over time, approaching an average of one breakthrough bleed every 2 years after 3 years of continuous use. rFVIIa is the preferred BPA for the treatment of breakthrough bleeding events; bleeding events treated with aPCC (a plasma-derived mixture of zymogens and activated coagulation factors the efficacy of which is primarily driven by the presence of prothrombin and FXa) carry an elevated risk of thrombotic microangiopathic and thromboembolic events if a cumulative dose of >100 U/kg/24 h is administered for longer than 24 h (REF.[159]). These observations have been attributed to a synergistic interaction between emicizumab and aPCC that results in greater than normal thrombin production[160]; patients treated for breakthrough bleeds should be monitored when repeated doses of aPCC are used. These thrombotic observations have not been reported with the use of emicizumab and rFVIIa alone[160]. Preferentially, the minimally required dose of either aPCC or rFVIIa should be used with emicizumab. National medical societies have published recommendations for the use of emicizumab and BPAs in patients with inhibitors[161].

The demonstrated efficacy of emicizumab in patients with haemophilia A with inhibitors has prompted fresh points of view on implementing ITI regimens in this patient population[162,163]. Indeed, with emicizumab available as a prophylactic option, the question of whether ITI should still be offered has been raised[164]. Given the length, cost and varying success of ITI protocols[165], emicizumab prophylaxis independent of ITI may represent an alternative approach to reduction of bleeding and improved quality of life in some patients with haemophilia A with inhibitors[163]. Nonetheless, inhibitor eradication remains an important goal; patients with inhibitors have higher mortality than patients without inhibitors, and the absence of inhibitor enables factor replacement options for patients who experience breakthrough bleeding, traumatic bleeds or who are undergoing surgery[162]. Inhibitor eradication through ITI also preserves the possibility for patients without inhibitors to some day receive a gene therapy-based cure for haemophilia, which might not be offered to patients with inhibitors[162].

Emicizumab prophylaxis during ITI to reduce bleeding and inflammation during tolerization has been hypothesized to potentially ease ITI treatment burden[163]; however, data regarding outcomes, ABRs and best dosing regimens of concomitant ITI regimens with emicizumab are limited. The Atlanta protocol uses an intermediate FVIII dose regimen in patients receiving emicizumab. This protocol shows promising initial results that include tolerization and lack of thrombotic events despite the concomitant use of rFVIIa, FVIII or antifibrinolytics to treat bleeding events[166]. A prospective observational study is planned.

Gene therapy

Non-insertional gene therapy trials using adeno-associated virus (AAV)-based vectors in haemophilia A and B have been reported[167], and interim results seem promising, with near complete eradication of both bleeding episodes and the need for factor replacement therapy with some protocols[168]. With non-insertional gene therapy products, the levels of the newly introduced factor may decrease with time; to avoid the need for future re-treatment, stable expression of clotting factor transgenes must be achieved. *F8* expression in the longest

running FVIII gene therapy trial, although still providing clinical benefit, has shown a decline over 4 years[169]. Another recent haemophilia A trial has demonstrated sustained *F8* expression for >2 years, which may suggest that durable *F8* transgene expression can be realized[170]. The use of *F9* Padua construct has improved FIX expression levels from such vectors, thus enabling lower vector doses to be used[171]. Clinical data from one haemophilia B trial demonstrate sustained *F9* expression 8 years after transgene delivery[172], whereas *F9* transgene expression in an earlier AAV-based trial was relatively short-lived, showing decreases after 4–6 weeks[173]. Cytotoxic immune responses against transduced hepatocytes that present AAV capsid peptides may contribute to the observed lack of persistent transgene expression[174]. AAV-mediated clotting factor gene expression levels and sustainability remain difficult to predict in individual patients; further research into AAV vector biology is needed to fully understand and optimize these promising approaches to haemophilia treatment.

Specific bleeding phenotypes

Severe bleeds, including intracranial haemorrhage or bleeding that may compromise the airway, require urgent treatment and hospitalization. Intracranial haemorrhage can lead to severe long-term neurological consequences or death. Patient recognition of head trauma can be difficult, as patients may experience slow or intermittent bleeding for several weeks following a minor head injury[175].

Bleeds into confined areas including the hip, calf, forearm and groin may compromise the neurovascular bundle and result in a compartment syndrome or aseptic necrosis of the femoral head. Treatment primarily involves aggressive haemostatic replacement; in extreme cases, a fasciotomy (a surgical procedure in which the fascia is cut) or joint aspiration may be required[175,176]. Iliopsoas bleeds may present with ambiguous symptoms, such as thigh, groin, testicular or back pain, resulting in difficult diagnosis; aggressive prolonged therapy and bed rest, followed by a period of physical therapy, are required for resolution[110].

Pseudotumours (that is, encapsulated progressive cystic swellings usually involving muscle and/or bone) are a consequence of inadequate bleed treatment, and are more common in resource-constrained settings[177]. They are more frequently observed in patients who have not recognized bleeding symptoms, have delayed treatment or do not have access to adequate therapy. Continued pseudotumour presence may result in bone erosion, neurovascular complications and disability. Pseudotumour treatment requires a skilled knowledgeable team, especially for surgical removal[177].

The classic debilitating long-term consequence of haemophilia not treated with regular prophylaxis is arthropathy with destruction of joint bone and cartilage, resulting from repeated blood deposition and subsequent inflammation in joints[178]. Repeated bleeding into the same joints, termed 'target joints', increases the risk of arthropathy; once joint damage has begun, the process is progressive and irreversible[179]. Interventions may include pain management (see below), radiosynovectomy (intra-articular injection of radioactive particles), surgical debridement, arthrodesis (bone fusion) and arthroplasty (reconstruction or joint replacement)[180–182]. Prophylactic regimens used after joint bleeding has occurred may slow arthropathic progression as may joint aspiration: an 11-year study noted that prophylaxis and prompt aspiration following all joint bleeds resulted in clinically normal joints[183]. Shorter times to joint bleed resolution, pain relief, range-of-motion recovery and resumption of school or work after joint aspiration have also been observed[184]. However, joint aspiration is not common practice: if performed, the procedure must be done using aseptic conditions with adequate haemostatic coverage[184].

Surgery

Non-emergency surgery in individuals with haemophilia requires evaluation and planning by a knowledgeable haemophilia team[182]. Dental care or interventions also require the involvement of the comprehensive haemophilia team to prevent bleeding, including delayed bleeding, anaemia or airway compromise that may occur with nerve block injections.

The need for additional haemostatic agents during dental and surgical procedures for patients on emicizumab prophylaxis is not fully defined. During minor procedures, some patients require additional haemostatic agents, whereas others do not[185]; this observation is similar to the finding that in mild haemophilia, outcome is patient specific. For major procedures and surgeries, use of other haemostatic agents is recommended, owing to the risk of complications that could result from substantial bleeding[161]. It should be noted that emicizumab can interfere with several standard coagulation assays[186]; alternative assays are required to measure coagulation parameters.

Pain management

Pain is a common and lifelong consideration for patients with haemophilia and contributes to disease burden. Thus, pain assessment and treatment is a vital aspect of improving outcomes and quality of life for individuals with haemophilia[187]. Pain in haemophilia can be either acute or chronic. Acute pain in haemophilia is typically the result of a bleeding episode, with joint bleeds accounting for 70–80% of bleeding episodes in severe haemophilia[83]. Thus, pain can potentially signal an active bleed in joints or other areas. Reported data from several studies indicate that 15% to >50% of adults with severe haemophilia experience chronic pain[188], which can stem from progressive joint degeneration caused by repeated bleeding into joint cavities.

Prompt treatment of acute bleeds is key for rapid bleeding control and pain reduction; additional medications may be needed to further control pain[83,189]. Although no randomized clinical trials have been performed to assess paracetamol (also known as acetaminophen) for treating pain in patients with haemophilia, paracetamol seems to be widely used for this purpose: a study of pain management approaches in Europe revealed that only paracetamol was used by all 22 haemophilia treatment centres surveyed, for both acute and

chronic pain[190]. Other analgesics used included strong opioids, cyclooxygenase-2 (COX2) inhibitors and NSAIDs[190]. A study of haemophilia treatment centres in the United States revealed that NSAIDS and paracetamol were the most commonly used medications for pain in patients with haemophilia[191]. Interestingly, patients have reported an analgesic effect when using factor replacement to manage pain; a potential psychological component contributing to pain relief in these instances has been suggested[192]. These collective observations underscore the current lack of standardized guidelines for pain management in haemophilia. Physical therapy programmes to prevent muscle atrophy, recover range of motion and alleviate pain in patients with haemophilia represent non-pharmacological interventions[187,189].

Quality of life

Quality of life (QoL) has been defined by the WHO as "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns"[193]. It is increasingly recommended to include QoL as an outcome measure in research and assessment of care in persons with haemophilia. Of note, QoL measures are included in the International Classification, Disability and Health (ICF) framework approved by the WHO in 2001 (REF.[193]). This framework is useful when considering the impact of bleed-related arthropathy in people with haemophilia (FIG. 3). A systematic review of the measurement properties of QoL instruments for people with haemophilia has been published[194]. There are two classes of QoL instrument: generic and disease specific. Generic instruments offer the possibility to compare QoL between different chronic conditions. Disease-specific, health-related quality of life (HRQoL) instruments enable collection of more information relevant to a specific disorder. In some situations, a combination of both classes of instrument is optimal.

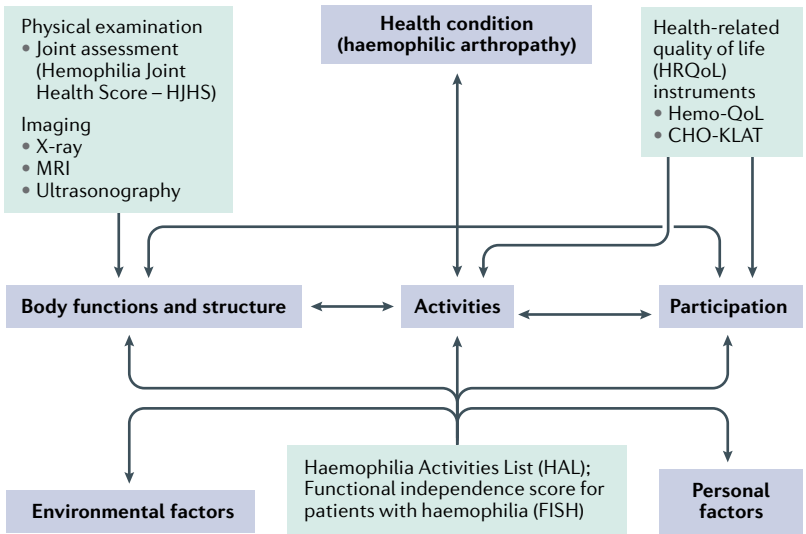

Fig. 3 | **Outcome measures in haemophilia.** Framework outlining outcome measures for use in the assessment of functioning, disability and health (with focus on musculoskeletal disease) in persons with haemophilia. Adapted with permission from REF.[228], World Health Organization.

HRQoL instruments for haemophilia

Development of haemophilia-specific QoL instruments requires several formal steps: item generation, item reduction and cognitive debriefing. Once developed, QoL and HRQoL instruments need to be validated, including cross-cultural validation in languages relevant to countries in which the instruments will be used. Cross-cultural validation requires that the instrument is formally translated from the parent language used to develop it into the language of interest, and that the translated instruments undergo formal cognitive debriefing. These steps are labour intensive and for some HRQoL tools inadequately completed. An ideal HRQoL instrument should have good measurement properties, that is, it should be reliable, valid and responsive to change.

PROMs and proxy-reported HRQoL tools

Well-designed and validated HRQoL instruments are an important part of the outcome measures 'tool kit' recommended for individuals with haemophilia, as endorsed by the recommended international standard outcomes set for persons with haemophilia[195]. A patient-reported outcome measure (PROM) is a measure reported by an individual, either self-reported or reported in an interview with a trained interviewer. It is generally accepted that children need to be older than 7 years of age to reliably complete a HRQoL questionnaire; for children younger than 7 years, a parent or guardian can act as proxy for their child. The value of HRQoL measures in people with haemophilia is increasingly recognized. However, there are concerns regarding the use of HRQoL scores to influence management decisions. HRQoL scores are not intended, and should not be used, to influence decisions regarding treatment of people with haemophilia on their own[196].

Currently available HRQoL instruments were developed in the era of SHL FVIII and FIX concentrates (BOX 2). In our experience, the CHO-KLAT version 2.0 questionnaire was not able to detect clinically meaningful differences between boys on long-term prophylaxis with very low spontaneous bleeding rates who were switched from a SHL to an EHL FVIII concentrate and who maintained very low spontaneous bleeding rates[197]. A possible explanation for this finding is that the current CHO-KLAT v2.0 questionnaire does not contain a sufficient number of questions that address the different burden of administration between SHL and EHL clotting factor concentrates, specifically the frequency of intravenous infusions needed to achieve an equivalent trough factor level. In an attempt to address this gap in measurement property of the CHO-KLAT v2.0 tool in the era of EHL clotting factor concentrates and non-factor haemostatic agents, a new version of the questionnaire has been developed[198]. In addition, currently available HRQoL instruments are not validated to measure the burden of illness on families of very young boys (<4 years of age) with moderate or severe haemophilia. This is an important gap in the available battery of proxy-reported outcome measures, as it is recommended by the WFH that programmes of primary prophylaxis in paediatric patients with haemophilia A or B be ideally started

Box 2 | **Health-related quality of life instruments for persons with haemophilia**

Children

- CHO-KLAT[229]
- Haemo-QoL[230]
- Haemo-QoL Index[231]
- 'Toddler Questionnaire'[232]

Adults

- Haemo-A-QoL[233]
- Haemo-QoL-A[234]
- Hemofilia-QoL[235]
- Hemophilia Well-Being Index[236]
- Hemolatin-QoL[237]

QoL, quality of life.

before age 3 years[83]. New tools such as the Hemophilia Family Impact (H-FIT) tool offer the potential to address this gap in proxy-reported outcome measures[199].

Outlook

Haemophilia therapy is undergoing a revolution, owing to a large emerging armamentarium of principles and products, including improved clotting factors, non-replacement therapy and gene therapy. The possibility of achieving zero bleeds and treating patients with inhibitors as well as achieving phenotypic cure seems realistic[1,10,200]. Even if progress has been made in the treatment of haemophilia, there are still several unresolved issues to address. The most important is probably how to achieve early accurate diagnosis and minimal treatment standards for all persons with haemophilia globally. There are many hurdles to overcome: sufficient availability of haemophilia treatment centres with diagnostic capacity is lacking in many low-income countries, and, therefore, many patients are not diagnosed. The WFH global survey shows a 10–20-fold higher prevalence of haemophilia in high-income countries than in some of the densely populated low-income countries, indicating variations in diagnostic capacity but also in treatment, as survival certainly has a strong effect on prevalence[5]. Development of care, including establishment of treatment centres and access to safe therapeutic agents, is perhaps the most urgent need for haemophilia. Humanitarian aid efforts by the WFH are important steps to provide more individuals with haemophilia with better care. Thus, in 2015, a prospective donation programme of 100 million IU of FVIII products per year over 10 years was established, and beneficial progress for the first year has been described[201]. In addition, specific focus needs to be directed towards female carriers and people with non-severe haemophilia. For both these populations, there are obvious gaps in terms of diagnosis, QoL and joint disease[19,202,203]. The new WFH Guidelines[83] are universally accessible and serve as an important tool to improve quality and equity of care globally. Finally, the introduction of national registries is essential to identify all patients and carriers. Such initiatives have been taken, and national registries are in place in countries with highly developed care as well as multinational registries for specific purposes (for example, the PedNet[204] and EUHASS[205] registries). The WFH has recently launched a World Bleeding Disorders Registry (WFH-WBDR).

Therapeutic options

The advent of FVIII and FIX products with improved pharmacokinetic profiles (the EHL products) offers the possibility, in patients with haemophilia A, to either extend intervals between injections, with decreased treatment-associated burden, or to maintain higher trough factor levels with the same frequency of infusions, whereas in patients with haemophilia B, it may be possible to achieve both of these objectives simultaneously. The end result should be improved bleeding protection and long-term maintenance of healthy musculoskeletal status. However, how the new products and clotting factors should be used remains unclear, in particular, how EHL concentrates would be of most benefit to the individual with haemophilia. We simply do not know how to dose FVIII or FIX in the most optimal way, irrespective of the performance of the concentrate. What we know is that treatment needs to be personalized, as pharmacokinetics, bleeding phenotype and lifestyle are different in each patient[206,207]. As the benefits of starting prophylaxis early seem to overshadow the benefits of dosing optimization later in life[208], personalizing the start of prophylaxis before joint disease develops should be prioritized, although the exact timing is difficult to determine, but at least one or two bleeds in the same joint should be avoided[209]. Personalized dosing should also take several aspects into account: pharmacokinetic profiles of infused factor concentrate, bleeding phenotype, treatment-associated burden, adherence and societal perspectives. An important tool to implement personalization is the use of population pharmacokinetics and Bayesian analysis[210]; several software programs such as the Web-Accessible Population Pharmacokinetic Service (WAPPS) and myPKFiT dosing tools are now available for use in the clinical setting. In the future, the hope is that personalized therapy can replace weight-based, fixed-dose prophylaxis regimens, but surveillance of long-term effects on outcomes of different prophylaxis dosing strategies needs to be implemented.

Non-replacement products may promote haemostasis independently of FVIII or FIX and can also be given subcutaneously, which facilitates injections compared with the intravenous route. Emicizumab has been on the market for some time and has shown excellent results with highly significant reduction in ABRs in patients with inhibitors, which are comparable to bleeding rates observed in persons with haemophilia without inhibitors on effective programmes of long-term prophylaxis (that is, of the order of 0–2 spontaneous index joint bleeds per year). However, thrombotic complications have been reported in connection with emicizumab treatment[7]. Hopefully this adverse event can be mitigated or avoided with knowledge of how to treat acute bleeds in these patients. A concern with non-replacement products, discussed especially in the context of FVIII, is that the inherent feedback mechanism balancing the clotting system is not in place[211].

Nonetheless, even if the primary target population for these products is patients with inhibitors, these agents can also be used in patients without inhibitors, as they are efficacious and easy to use[8,212]. Anti-TFPI monoclonal antibodies are under investigation in clinical trials and, if successful, they could also be useful for FIX deficiency. Of note, however, to date, two out of three anti-TFPI molecules have been associated with severe thrombotic complications that led to premature interruption or pause of the clinical trial programme[213,214]. There are relevant issues with these novel products, as they have not been tested in randomized studies against replacement factor therapy. Such studies are difficult to design, and especially long-term follow-up of joint disease outcomes would be a substantial practical hurdle, as joint disease has a progression that can last many years. As the haemostatic capacity of these products in plasma samples cannot directly be compared with assayed levels of FVIII or FIX, uncertainties may arise regarding effectiveness to prevent subclinical bleeds (for example, in connection with minor trauma), especially as high concentration peaks like those observed after infusion with FVIII or FIX are not achieved. The question is whether practical issues with administration of new products have priority over known outcomes with replacement therapy. Such questions are extremely important to solve, as treatment during the first years of life may determine QoL later in life, as even a few joint bleeds may elicit progressive musculoskeletal destruction over years[208]. More data are needed regarding adverse events with new replacement and non-replacement haemostatic agents, and individuals who are offered these novel therapies need to be educated about the potential risks, benefits and alternatives very clearly before prescription.

Finally, gene therapy for haemophilia seems poised to become a reality soon in the clinical setting. Trials are promising for both haemophilia A and haemophilia B[10]. Many aspects need to be addressed when introducing gene therapy on a wider basis, such as to whom it should be offered, safety issues and economic considerations, but the possibility of phenotypically curing haemophilia is within sight. Needless to say, the potential of gene therapy to maintain appropriate factor levels and sustained expression over many years has the great potential benefit to reduce the patient burden of injections and living with uncertain haemostasis.

Inhibitors

Development of inhibitors today is the main challenge for haemophilia treatment. Research has progressed our understanding of the underlying immunological and clinical aspects of inhibitors[215], and development of non-replacement therapies such as factor VIII inhibitor bypassing agent (FEIBA), rFVIIa and more recently emicizumab has improved the possibilities for treating and preventing bleeds in these patients. Emicizumab has been established as the preferred haemostatic agent for prophylaxis in both children and adults with high-titre inhibitors[216]. Where emicizumab is available, the use of FEIBA or rFVIIa can no longer be supported for long-term prophylaxis.

Much focus has been devoted to risks of inhibitor development associated with genetics, type of concentrate and dosing aspects[30,215,217–219], but still a high risk of inhibitor incidence remains. An important goal for treatment is to eradicate inhibitors, and several protocols have been developed, often based on the original Bonn protocol[220]. A fraction of patients are resistant to ITI[221], and further clinical exploration of protocols with combined immunosuppression is required for this population; it is hoped that new non-replacement therapies will further improve the situation for this patient group.

Published online: 24 June 2021

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Author contributions

Introduction (E.B.); Epidemiology (K.F.); Mechanisms/pathophysiology (D.P.H. and D.S); Diagnosis, screening and prevention (M.E.M.); Management (A.D.S.); Quality of life (V.B.); Outlook (E.B.); Overview of Primer (E.B.). All authors provided critical evaluation of the complete manuscript.

Competing interests

E.B. has received grants and/or research support from Bayer, CSL Behring, Shire and Sobi/Bioverativ and honoraria and/or consultation fees from Bayer, Octapharma and Shire/Takeda. The Van Creveldkliniek has received speaker's fees from Bayer, Baxter/Shire, Sobi/Biogen, CSL Behring and Novo Nordisk; has performed consultancy for Bayer, Biogen, CSL Behring, Freeline, Novo Nordisk, Roche and Sobi; and has received research support from Bayer, Baxter/Shire, Novo Nordisk, Pfizer and Biogen for work done by K.F. Queen Mary University London has received research grants from Bayer, Octapharma and Takeda for work performed by D.P.H. He or his institution have received speaker or consultancy honoraria from Bayer, BioMarin, Biotest, Grifols, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Spark Therapeutics, Sobi, Takeda and UniQure. M.E.M. has acted as paid consultant, adviser or speaker for Bayer Healthcare, BioMarin, Catalyst, CSL Behring, Grifols, Kedrion, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sobi, Spark Therapeutics and Takeda. D.S. is Chair of EAHAD Physiotherapy Committee, received research grant funding from the National Institute for Health Resarch (NIHR), Novo Nordisk, Pfizer, Roche, Sobi and acted as paid adviser or speaker for Bayer, Pfizer, Sobi, Roche and Takeda. V.B. is Chair of the International Prophylaxis Study Group (the IPSG), which is funded by grants from Bayer Healthcare, Bioverativ (now Sanofi/Genzyme), Novo Nordisk, Pfizer, Shire (now Takeda) and Spark Therapeutics to the Hospital for Sick Children Foundation, Toronto, Canada. He has received fees for participation in education events and advisory boards sponsored by Amgen, Bayer Healthcare, Novo Nordisk, Pfizer, Roche and Shire (now Takeda), and for participation in data safety monitoring boards for Octapharma and Takeda. He is recipient of investigator-initiated grants from Bayer Healthcare, Bioverativ (now Sanofi/Genzyme) and Shire (now Takeda). A.D.S. has served as consultant for Genentech, Roche, Novo Nordisk, BioMarin, Bioveritiv, Sanofi, ProMetic Bio Sciences, Kedrion, Sigilon and Takeda. She has received research funding from the above plus Agios, OPKO, Global Bio Therapeutics, Sangamo, Sigilon, Octapharma and Novartis.

Peer review information

Nature Reviews Disease Primers thanks C. Hermans, K. Kavakli and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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